REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and the following comments.

I. Status of the Claims

Claims 2-13 are cancelled without prejudice or disclaimer thereof. Claim 1 have been amended and claims 14-16 have been added with exemplary support in the original specification as detailed in the table below.

Claim	Exemplary Support
1	Paragraph bridging pages 4 and 5; page 6, first and second full paragraphs; page 10; page 12; page 13, first full paragraph; figure 1
14	Page 5; page 7; page 15, first full paragraph
15	Page 24, last full paragraph
16	Page 5, first full paragraph; page 6, last full paragraph

Because no new matter is introduced, Applicants respectfully request entry of this amendment. Upon entry, claims 1 and 14-16 will be pending.

II. Claim Objections

Claims 4-8 are objected to for improper multiple dependency. Claims 4-8 have been cancelled thereby obviating the basis of the objection.

III. Rejection of Claims under 35 U.S.C. §112, second paragraph

Claims 1-8 are rejected for allegedly being indefinite. Specifically, claim 1 is rejected for the recitation of "a single measurement," and "quinone dye is not formed." Claim 1 have been amended to delete the recitations for greater clarity. Claims 2-8 have been cancelled thereby rendering the rejection moot.

IV. Summary of the Claimed Invention

The claimed invention relates to an assay method for quantifying low density lipoprotein (LDL) and total cholesterol (including chylomiron (CM), very low density lipoprotein (VLDL), LDL and high density lipoprotein (HDL)) in a biological sample.

The method entails a first step of adding a first reagent to the biological sample, such that hydrogen peroxide is generated from all lipoproteins but LDL, including CM, VLDL, and HDL. Due to the absence of peroxidase in the first reagent, the hydrogen peroxide generated in the first step will accumulate rather than being converted into a quinone dye. This is reflected in figure 1 of the specification as the reading of absorbance before the addition of a second reagent being at a baseline level.

In the second step of the claimed method, a second reagent, which contains peroxidase, is added to the sample. Peroxidase reacts immediately with the hydrogen peroxide accumulated in the first step such that a quinone dye is rapidly generated. As demonstrated by figure 1, a reading of absorbance taken immediately after addition of the second reagent represents the amount of cholesterol other than LDL in the sample. Because the second reagent also contains enzymes and surfactants that act on LDL, additional hydrogen peroxide is generated from LDL and converted to a quinone dye. As such, a reading of absorbance taken in five minutes after addition of the second reagent represents the amount of total cholesterol in the sample. The amount of the LDL is deduced from the difference of two readings.

The claimed invention is superior to the conventional method in the aspect that spontaneous color development during the first step is reduced thereby producing more accurate readings because the first reagent is stabilized due to absence of peroxidase. See specification, at page 3, lines 23-25.

V. Rejection of Claims under 35 U.S.C. §102

A. Matsui

Claims 1-8 are rejected under 35 U.S.C. §102(a) for alleged anticipation by Matsui, *Japanese J. Clin. Lab. Automation* 28(4): 380 (August, 2003) ("Matsui"). Applicants respectfully traverse the rejection.

Unlike the claimed invention, in which the hydrogen peroxide generated by lipoproteins other than LDL is not converted into a quinone dye until addition of a second reagent, Matsui's method entails converting CM, VLDL, and HDL into a quinone dye and taking a measurement of absorbance **before** adding a second reagent. See Matsui, figure 1, and the section entitled "Measurement Principles" (relevant passage excerpted below, with emphasis added):

> *Cholesterols in lipoproteins other than LDL contained in the blood are allowed to react in a first reaction, thereby* ***resulting in their detection in the form of absorbance****. Subsequently, LDL-C is allowed to react in a second reaction, thereby resulting in its detection.*

In sharp contrast, no reading of absorbance is taken until additional of the second reagent in the claimed invention.

B. Nakamura

Claims 1-8 are rejected under 35 U.S.C. §102(b) for alleged anticipation by U.S. Patent No. 6,057,118 to Nakamura et al. ("Nakamura"). Applicants respectfully traverse the rejection.

Nakamura is directed to a method for quantitatively determining the amount of LDL in a sample. Specifically, a specific surfactant which dissolves HDL and VLDL but retards the reaction of LDL is added; after termination of the first reaction, the amount of LDL is measured. See Nakamura, column 2, lines 29-41. Nakamura has no teaching or suggestion as to how to

measure the total cholesterol in the sample. In sharp contrast, the claimed invention allows quantification of lipoproteins other than LDL, LDL and total cholesterol in a sample.

C. Kanno

Claims 1-8 are rejected under 35 U.S.C. §102(b) for alleged anticipation by Kanno et al., Current Therapy 16(a): 146-150 (1998) ("Kanno"). Applicants respectfully traverse the rejection.

Similar to Nakamura, Kanno does not teach or suggest how to quantify total cholesterol but only measures the amount of LDL in a sample. Kanno describes "eliminating" lipoproteins other than LDL by producing a ***colorless compound*** and then LDL is converted to a ***colored compound***, the amount of which is quantified. As such, Kanno fails to teach or suggest a method for quantifying total cholesterol. The relevant passage of Kanno at page 147 is reproduced below (emphasis added):

> *. . . Specifically, surfactant 1 used in step 1 causes structural changes in chylomicron, VLDL, and HDL, and cholesterols contained in such lipoproteins can be subjected to the enzyme reactions . . . in the first reaction. Hydrogen peroxide produced during the reaction is* ***eliminated as colorless reaction product*** *by peroxidase and 4-aminoantipyrine. In the second reaction of step 2, addition of surfactant 2 results in the structural changes in LDL, which was not involved in the reaction of step 1 and remained, and can be subjected to the enzyme reactions. . . . since a color-developing agent, DSBmT, is added in step 2, hydrogen peroxide generated from LDL-C is subjected to* ***a bluish-purple color*** *reaction, and* ***LDL-C is then quantified****.*

D. Miki

Claims 1-8 are rejected under 35 U.S.C. §102(b) for alleged anticipation by U.S. Patent No. 5,925,534 to Miki et al. ("Miki"). Applicants respectfully traverse the rejection.

Miki's disclosure is similar to that of Matsui. Specifically, Miki describes that a first measurement of absorbance is obtained following addition of a first reagent solution, and then a

second measurement of absorbance is obtained following addition of a second reagent solution. See Miki, column 3, lines 29-34. This protocol is distinguishable from the claimed invention, in which no reading of absorbance is taken until the second reagent is added.

Because none of the cited references teaches each and every aspect to anticipate the claimed invention, Applicants respectfully request withdrawal of all rejections under Section 102.

VI. Rejection of Claims under 35 U.S.C. §103(a)

Claims 1-8 are rejected for allegedly being obvious over Matsui, Nakamura, Kanno and Miki, either alone or in combination. Applicants respectfully traverse the rejection.

The Examiner has yet to establish a *prima facie* case of obviousness by clearly articulating how the claimed invention is obvious over the combined teachings of the cited art and why one skilled in the art would have any reason to combine the cited art.

None of the cited references alone renders the claimed invention obvious. As discussed supra, the cited references fail to meet all claim limitations by failing to suggest a method for measuring the total cholesterol in a sample or a method of taking readings of absorbance only after addition of the second reagent. Accordingly, withdrawal of the rejection under Section 103(a) is warranted.

CONCLUSION

Applicants believe that the present application is now in condition for allowance and respectfully request a favorable indication to this effect. The Examiner is invited to contact the undersigned by telephone if a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by the credit card payment instructions in EFS-Web being incorrect or absent, resulting in a rejected or incorrect credit card transaction, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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